Effect of Husk Characters on Resistance to Corn Earworm (Lepidoptera: Noctuidae) in High-Maysin Maize Populations

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ABSTRACT Two maize (*Zea mays* L.) breeding populations with very high concentrations of maysin, a silk-expressed flavone glycoside, were tested for their ability to resist ear damage by the corn earworm, *Helicoverpa zea* Boddie, under field conditions. Tests were conducted in 2000 and 2001 at multiple locations in Georgia. The high maysin populations, EPM6 and SIM6, as well as resistant and susceptible checks, were scored for silk-maysin content, *H. zea* damage, and husk characters. In 2000, there was a negative correlation between husk tightness and earworm damage at three of five locations, while there was no significant correlation between damage and maysin content at any location. In 2001, EPM6 and SIM6 had approximately ten times the maysin content of the low-maysin control genotypes; nevertheless, earworm damage to EPM6 and SIM6 was either greater than or not significantly different from the low-maysin genotypes at all locations. The resistant control genotype, Zapalote Chico, had significantly less earworm damage than EPM6 and SIM6 for both years at all locations. The results of this study highlight the importance of identifying and quantifying husk and ear traits that are essential to *H. zea* resistance in maize.

KEY WORDS *Helicoverpa armigera*, *Helicoverpa zea*, husk tightness, husk wrap, host plant resistance, *Zea mays*

THE CORN EARWORM (*Helicoverpa zea* Boddie) is a major pest of both field and sweet corn in the United States. *H. zea* moths lay eggs on fresh corn silks and upon hatching the larvae pass through the silks into the ripening ear, often feeding on silk tissue along the way. Earworm larvae are difficult to control with insecticide sprays because they spend so little time outside the ear. In major sweet corn producing areas such as southern Florida, heavy earworm pressure can force growers to spray insecticides >30 times to produce a single uninfested crop (Lynch et al. 1999a). Nationwide, field corn is commonly left untreated because of the low cash value of the crop. This can result in heavy earworm damage, which promotes infection by the aflatoxin-producing fungus *Aspergillus flavus*, especially in the southeastern states (McMillian et al. 1985). Aflatoxin contamination severely reduces the market value of grain corn.

Host-plant resistance (HPR) is a promising tool for controlling *H. zea* on corn because larvae cannot escape control by simply burrowing into the ear. Transgenic HPR, most often employing insecticidal proteins derived from *Bacillus thuringiensis* (Bt), has proven effective in early trials (Lynch et al. 1999a, b). However, transgenic crops have been the subject of worldwide controversy (Wolfenbarger and Phifer 2000, Gura 2001) and may not be welcome in all markets. In addition, the development of pest insect populations with resistance to Bt toxins is a concern (Gahan et al. 2001, Palumbi 2001).

Some maize genotypes produce a flavone glycoside in their silks called maysin that is toxic when ingested by *H. zea* larvae (Waiss et al. 1979, Snook et al. 1994). The presence of this chemical in the silks is auspicious for HPR purposes because female *H. zea* moths preferentially lay their eggs on corn silks and hatching larvae often feed on silks as their first meal. Thus, for maysin-producing genotypes, larval exposure to maysin can be high under normal field conditions. Widstrom and Snook (2001) have developed and released two maize populations, EPM6 and SIM6, that have approximately ten times the maysin concentration necessary to reduce *H. zea* larval growth by half compared with larvae fed diets without maysin (Wiseman et al. 1992).

Tight husks have been shown to reduce earworm damage in corn field trials (Wiseman et al. 1977, Archer et al. 1994), presumably by acting as a physical barrier to entry into the developing ear. It is possible that tight husks could enhance silk-based HPR against *H. zea* by forcing larvae to eat through maysin-containing silks to reach the developing ear.

The objectives of this study were to test the field efficacy of maysin-based HPR in EPM6 and SIM6 and to assess the relative effects of maysin content and

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husk characters on resistance to *H. zea* in these two populations and Zapalote Chico, a resistant check.

Materials and Methods

Plant Materials. Four genotypes were tested in 2000 and five were tested in 2001. In 2000, the genotypes were 'Zapalote Chico 2451# (P) C3′ ("ZC"), a dent population selected from the CIMMYT 'Oaxaca 35′ collection; 'Stowell's Evergreen' ("SEG"), a commercial sweet corn variety; and EPM6 and SIM6, two dent populations selected for high concentrations of maysin (Widstrom and Snook 2001). In 2001, the genotypes tested were ZC; EPM6; SIM6; 'Pioneer 3369A' ("P3369A"), a commercial dent hybrid; and TL563, a population generated from selfed F2 plants from the cross ZC x Mp313E that were selected for low maysin, early maturity, and tight husks.

ZC was included in the experiment as a resistant check in both 2000 and 2001. It is known to have a moderately high maysin concentration, very tight husks, and good field resistance to CEW (Wiseman and Widstrom 1992, Snook et al. 1993). In 2000 SEG was included as a susceptible check. It is known to have low maysin and loose husks (Wiseman and Widstrom 1992). In 2001, two dent corn genotypes were included as low-maysin checks in place of the sweet corn, SEG, to eliminate any possible larval bias toward either dent or sweet kernels. The two new genotypes, P3369A and TL563, are both known to have relatively low maysin content but differ with regard to husk traits (P3369A: Wiseman and Isenhour 1992; TL563: unpublished data).

Field Plots and Locations. Plots were 6-m long and 4 rows wide with 91 cm between rows. Four replicates were planted in a randomized complete block design at each of four Georgia locations, spanning three major land resource areas (Blue Ridge, Southern Piedmont, and Southern Coastal Plain) that cover 70% of the state. In 2000, plots were planted 27 March at the University of Georgia Southeast Branch Experiment Station in Midville; 19 April at the Belflower Farm in Tifton, with a second planting in Tifton on 16 May; 25 May at the University of Georgia, Mountain Branch Experiment Station in Blairsville; and 30 May at the University of Georgia Plant Sciences Farm near Athens. In 2001, P3369A, SIM6, and TL563 were planted 10 d earlier than EPM6 and ZC at each location to synchronize maturity of all genotypes. This facilitated data collection and ensured equal exposure to CEW oviposition at silking. P3369A, SIM6, and TL563 were planted 09 April at the University of Georgia Southwest Branch Experiment Station in Plains, 11 April at Tifton, 10 May at Blairsville, and 11 May at Athens. EPM6 and ZC were planted 19 April at Plains, 20 April at Tifton, 21 May at Blairsville, and 22 May at Athens.

Silk Collection and Maysin Analysis. Fresh silks were collected from the second ears of ten tagged plants chosen from the middle two rows of each plot. Silks were gathered from second ears to avoid interfering with husk and insect-damage data that would be gathered later from the first ears. Comparison of second-ear maysin concentration as an inference of relative first-ear maysin content has been previously established (Wiseman et al. 1993). The criteria for plant selection were the presence of a second silking ear and enough overall plant vigor to ensure a well-developed first ear suitable for subsequent data collection. Silks were collected on ice, weighed and steeped in methanol for at least 14 d at −10°C to extract maysin. Maysin content was analyzed by reverse-phase high performance liquid chromatography with a methanol gradient as described by Snook et al. (1994).

Husk Tightness, Husk Wrap, and Husk Coverage Data. Husk characters were measured for the first ear of each tagged plant 10 to 14 d after silking. Husk tightness was measured in 2000 using a 0 to 5 scale (Wiseman et al. 1977, Wiseman and Isenhour 1992) that combines the coverage of the ear tip and the tightness of the husk around the ear into a single subjective score. In 2001, a new rating system was developed to more precisely identify the husk characters that may be associated with earworm resistance. Husk tightness around the ear ("husk wrap") and husk coverage of the ear tip ("husk coverage") were scored independently. In this rating system a 1–5 score was given for husk coverage where 1 = exposed kernels, 2 = open end but no kernels exposed (i.e., only silks covering ear tip), 3 = tip covered by husks, 4 = silk channel present, and 5 = long silk channel present. A separate 1 to 5 score was given for husk wrap where 1 = very loose, 2 = loose, 3 = moderate tightness, 4 = tight, and 5 = very tight.

Earworm Damage Ratings. Damage ratings were collected after natural infestation of *H. zea.* Earworm feeding on first ears of tagged plants was measured as centimeters of penetration along the ear, 18 to 21 d after flowering, using the Revised Scale developed by Widstrom (1967).

Data Analysis. Data were analyzed by analysis of variance (ANOVA) and correlations were made using Pearson's correlation coefficient (PROC CORR) (SAS Institute 1989). Means were separated by Waller Duncan k-ratio *t*-test (Waller and Duncan 1969).

Results and Discussion

In both 2000 and 2001, the silk-maysin concentrations of EPM6 and SIM6 were higher than those of any other entry at all locations (see Figs. 1a and 2a). Indeed, to our knowledge these two populations have higher silk-maysin concentrations than any other maize genotype. However, these higher maysin concentrations were not correlated with reduced ear damage in either year at any location.

In 2000, earworm damage to ZC was lower than damage to either EPM6 or SIM6 at all locations, while damage to SEG was either higher than or not different from EPM6 or SIM6 at all locations (Fig. 1c). ZC, which had the tightest husks at every location (Fig. 1b), had the lowest damage ratings at every location (Fig. 1c), while SEG had the loosest husks (Fig. 1b) and the most damage (Fig. 1c) at all locations. Husk

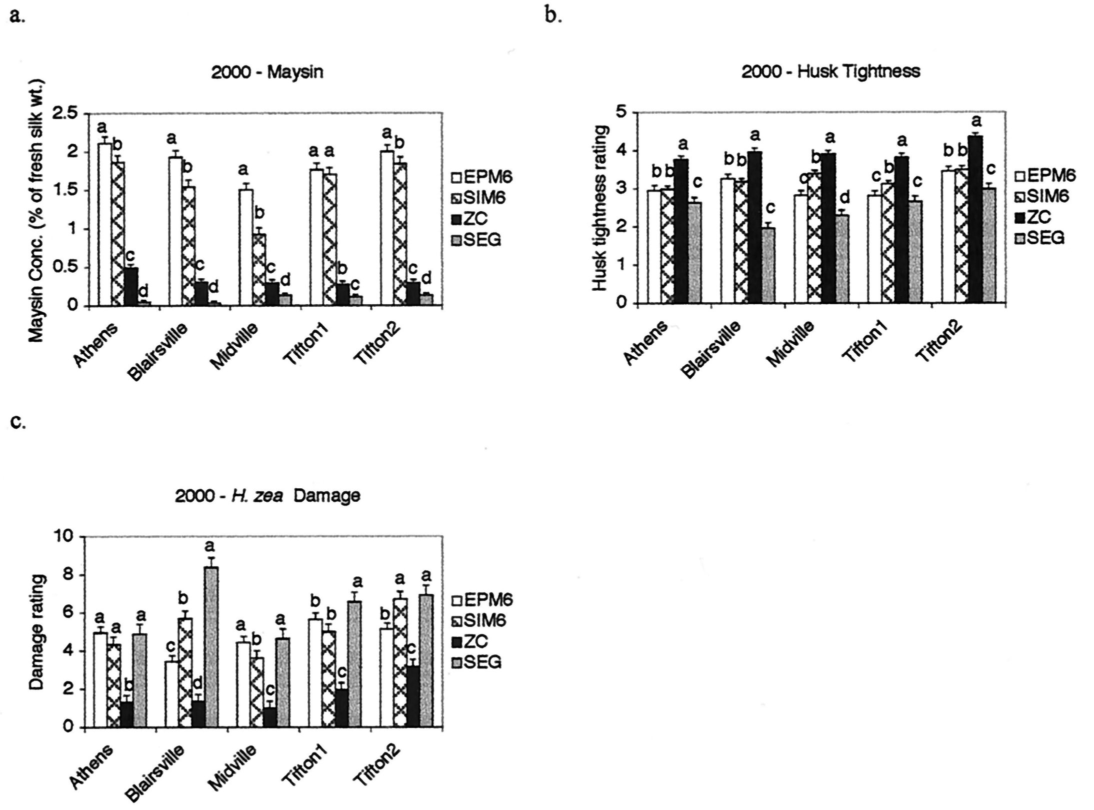

Fig. 1. Silk-maysin concentrations (a), husk tightness ratings (b), and ear damage by *H. zea* (c) for four maize genotypes at five locations in Georgia in 2000. Bars represent means and vertical lines represent standard errors of the means. Presence of the same letter above two bars indicates no significant difference between genotype means at that location.

tightness was negatively correlated with *H. zea* damage at three of five locations in 2000 (Table 1).

In 2001, *H. zea* damage to EPM6 and SIM6 was either more severe than or not significantly different from all other genotypes at all locations (Fig. 2 d). Husk wrap scores for both EPM6 and SIM6 were either lower than or not significantly different from all other genotypes at all locations with the single exception that SIM6 had higher husk wrap than P3369A at Plains (Fig. 2b). There was a highly significant negative correlation between husk wrap and *H. zea* damage in Athens in 2001 but not at any other locations (Table 2). SIM6 husk coverage scores were the highest at all locations while EPM6 husk coverage was significantly lower than SIM6 husk coverage at every location (Fig. 2c).

Table 1. Correlation of corn earworm (*H. zea*) damage with husk tightness score for maize entries grown at five locations in Georgia in 2000

Location	Pearson correlation coefficient (n = 4)	P-value
Athens	−0.956	0.044
Blairsville	−0.958	0.043
Midville	−0.902	0.098
Tifton 1	−0.987	0.013
Tifton 2	−0.911	0.089

There was no correlation between husk coverage and ear damage at any location (data not shown). Husk wrap and husk coverage scores were summed to approximate the husk tightness score that was used in 2000 but there was no correlation between the sum (husk wrap + husk coverage) and *H. zea* damage at any location (data not shown).

The results from both 2000 and 2001 show that expression of high concentrations of maysin in corn silks is not enough by itself to prevent or reduce ear damage by *H. zea*, despite the severe growth reduction that EPM6 and SIM6 silks cause to *H. zea* larvae in the laboratory (data not shown). It could be that *H. zea* larvae in the field are not feeding on the high-maysin silks of EPM6 or SIM6 before they reach the developing ear. It is possible that the silks of EPM6 and SIM6 have an antixenotic effect, because of extreme maysin concentrations or to some other factor, which causes larvae to avoid feeding until they have crawled past the silks to the developing kernels. ZC silks may lack this repellent effect and would thus be fed upon by the larvae. Such feeding would contribute to resistance because ZC silks have maysin concentrations high enough to significantly reduce *H. zea* larval growth (Wiseman et al. 1985, 1992, Snook et al. 1993, 1994). Experiments measuring the relative preference by *H.*

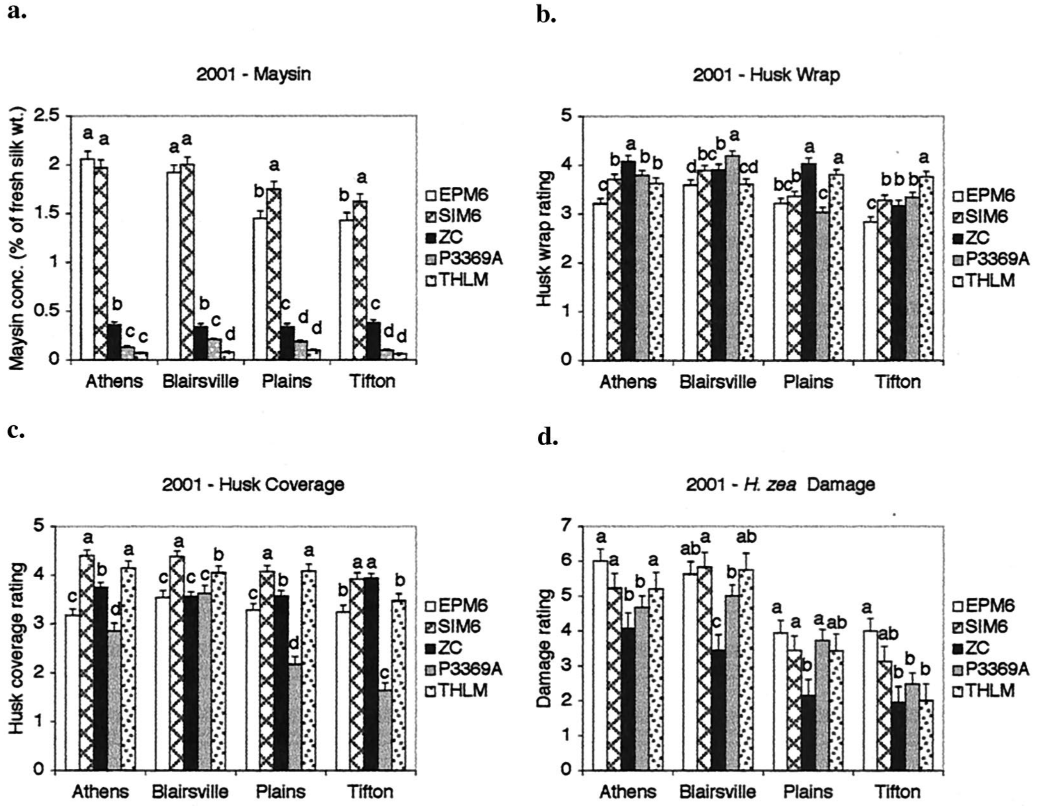

Fig. 2. Silk-maysin concentrations (a), husk wrap ratings (b), husk coverage ratings (c), and ear damage by *H. zea* (d) for five maize genotypes at four locations in Georgia in 2001. Bars represent means and vertical lines represent standard errors of the means. Presence of the same letter above two bars indicates no significant difference between genotype means at that location.

zea larvae for silks from EPM6, SIM6, ZC, and a variety of other maize genotypes, either containing or lacking maysin, will be necessary to test for the presence of such an antixenotic effect.

The negative correlations that were detected between ear damage and husk tightness in 2000 and between ear damage and husk wrap in 2001 are not surprising because previous studies have found similar correlations (Wiseman et al. 1977, Archer et al. 1994). These results suggest that the *H. zea* larvae were often prevented by the tight ZC husks from reaching the developing kernels without eating a path for themselves through the maysin-containing ZC silks. This could explain why ZC had the lowest damage ratings at all locations in both years (Figs. 1c and 2d). The looser husks of EPM6 and SIM6 may have allowed larvae to avoid feeding on silks by crawling directly to the developing kernels. An observational study of neonate *H. zea* behavior on silking ears of these and other genotypes should be done to investigate larval behavior.

Zapalote Chico has been used as a resistant control in studies of maize resistance to *H. zea* for many years because of its remarkable insect resistance under field conditions as well as the antibiotic effect of its silks in laboratory assays (Wiseman et al. 1977, 1985, Wiseman and Widstrom 1992, Abel et al. 2000). Maysin was identified as the principal chemical involved in this antibiosis in 1979 (Waiss et al. 1979) and a breeding program to increase silk-maysin concentrations was begun soon afterwards. EPM6 and SIM6 represent the fruits of that successful breeding effort (Widstrom and Snook 2001). However, the results of this study make it clear that high levels of maysin alone are not enough to provide effective resistance to *H. zea.*

The most striking physical feature of the highly-resistant ZC plant is its husk. The ZC ear is very tightly wrapped in a large number of tough husk leaves. The importance of this husk tightness to maize resistance to *H. zea* has been known for some time. There has also

Table 2. Correlation of corn earworm (*H. zea*) damage with husk wrap score for maize entries grown at four locations in Georgia in 2001

Location	Pearson correlation coefficient ($n = 5$)	P-value
Athens	−0.976	0.004
Blairsville	−0.386	0.521
Plains	−0.836	0.078
Tifton	−0.728	0.164

been speculation about the role of other ZC ear traits, such as rapid silk dessication, in resistance to *H. zea* (Wiseman et al. 1977). However, the precise husk or ear characters that are most essential to resistance to *H. zea* have not yet been elucidated. Identification and introgression of these characters, in combination with the high maysin concentrations exhibited by EPM6 and SIM6, will accelerate the development of improved maize genotypes with resistance to CEW.

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